Kidney International, Vol. 38 (1990), pp. 12–18

Effects of recombinant human erythropoietin on HLA sensitization and cell mediated immunity

PAUL C. GRIMM, LEVANA SINAI-TRIEMAN, NEAL M. SEKIYA, LINDA S. ROBERTSON, BRENDA J. ROBINSON, RICHARD N. FINE, and ROBERT B. ETTENGER

Pediatric Histocompatibility Laboratory and Division of Pediatric Nephrology, UCLA School of Medicine Los Angeles, California, USA

Effects of recombinant human erythropoietin on HLA sensitization and cell mediated immunity. Highly presensitized patients wait longer for a renal allograft than unsensitized patients and have a poorer allograft survival rate. Repeated blood transfusions have been implicated in the induction and maintenance of sensitization. To determine the effect of recombinant human erythropoietin (rHuEPO) therapy on five transfusion dependent, highly sensitized adolescents on dialysis, we serially measured percentage panel reactive antibody (%PRA) levels, titers of identifiable discrete anti-HLA Class I antibody specificities, and non-specific indices of cellular immunity before and following initiation of rHuEPO therapy. Although four of the five patients had previously rejected at least one renal allograft, the removal of chronic antigenic stimulation from blood transfusions led to a marked reduction in anti-HLA antibody titers to recognizable private and public specificities ($P < 0.001$) and a reduction of mean %PRA from 80% to 56% ($P < 0.05$). Each patient demonstrated a reduction of two or more dilutions to at least two anti-HLA antibody specificities. A control group of five patients matched for age, transfusion dependence and sensitization status demonstrated no change during a comparable time interval. PHA responsiveness decreased significantly in the rHuEPO group whereas autologous and allogenic mixed lymphocyte response, spontaneous blastogenesis and T-cell subsets did not. These data indicate that in highly sensitized dialysis patients rHuEPO may lead to decreased sensitization, shorter waiting time on dialysis and possibly improved allograft survival rates.

In patients on dialysis, repeated exposure to foreign HLA antigens can result in a highly presensitized state. Humoral presensitization is measured by reacting patient serum against a panel of T lymphocytes from normal donors using the complement dependent cytotoxicity (CDC) technique [1]. The result is expressed as the percentage panel reactive antibody (%PRA). For more than a decade, highly presensitized patients have made up an ever-increasing proportion of those awaiting renal transplantation [2]. A potential recipient's serum must be devoid of antibody against the donor's Class I HLA antigens at the time of transplantation in order to avoid hyperacute rejection [3]. The waiting time for such a suitable crossmatch-negative donor kidney increases linearly with the degree of sensitization [4]. The median waiting time is more than four years if the PRA is > 95% [5].

Received for publication March 23, 1990
and in revised form April 20, 1990
Accepted for publication April 24, 1990

Presensitization also adversely impacts allograft survival. First transplants with a PRA > 50% have a one year graft survival rate 8% lower than unsensitized patients, while re-transplants with a PRA > 10% have a 10% lower graft survival rate than similar unsensitized recipients [5]. This may be due to the adverse effects of the anti-HLA antibodies upon allograft outcome. Alternatively, sensitization could be an index for augmented T cell-mediated immune activity. A number of studies have suggested that allograft outcome is affected by the strength of the non-specific cell-mediated immune response [6, 7]. High levels of sensitization could thus signify a strong immune-responder status.

Patients may be sensitized by three mechanisms: prior pregnancy; previous allograft or blood transfusions. The relative contribution of each of these mechanisms to the initiation and maintenance of sensitization is controversial. Alone, blood transfusions may be less important [8], but following a failed transplant [2, 9] or a previous pregnancy [8], blood transfusions take on a greater importance in initiating sensitization. What is not clear, however, is the contribution of continuing blood transfusions to the maintenance of the sensitization.

Recently, recombinant human erythropoetin (rHuEPO) has been shown to reverse the anemia of chronic renal failure [10]. It appears that the need for blood transfusions may be totally obviated by rHuEPO in many dialysis patients. While the avoidance of blood transfusion should eliminate initiation of anti-HLA sensitization in patients without a previous pregnancy or prior allograft, the effect that such avoidance would have on an established state of presensitization is unknown. In particular, it is not clear what effect rHuEPO treatment and subsequent transfusion avoidance would have on the degree of anti-HLA sensitization in patients with established levels of presensitization, although Braun et al noted that rHuEPO therapy facilitated only some highly sensitized patients to experience a decrease in %PRA [11]. There are no studies on the effect of rHuEPO therapy and transfusion avoidance on the T cell immune response in dialysis patients with established anti-HLA sensitization.

We recently performed a preliminary study of rHuEPO treatment in five highly presensitized adolescents undergoing continuous cycling peritoneal dialysis (CCPD) [12]. Because of the paucity of data concerning transfusion elimination in highly presensitized dialysis patients, we prospectively studied both the humoral and T cell-mediated immune responses that appear

Table 1. rHuEPO patient data

Patient no.	Age *years*	Primary renal disease	Transfusions Prior	Transfusions During rHuEPO	Previous transplants	Outcome
I	18.5	MCD	23	0	1	Rejected at 8 yrs
II	12.5	FSGS	77	0	0	—
III	18.5	Chronic Gn	45	0	2	Rejected at 1 mo & 4 mo
IV	12	FSGS	28	0	1	Rejected at 6 yrs
V	18.5	RPGN	33	1	1	Rejected at 2 yrs
Mean			41			

Abbreviations are: MCD, medullary cystic disease; FSGS, focal segmental glomerulosclerosis; RPGN, rapidly progressive glomerulonephritis.

Table 2. Control patient data

Patient no.	Age *years*	Primary renal disease	Transfusions Prior	Transfusions During study (*no./year*)	Previous transplants	Outcome
I-C	15	Chronic GN	36	5	2	Rejected 3 mo & never functioned
II-C	13	IPKD	31	12	0	—
III-C	17	Alport's syndome	25	8.4	1	Never functioned
IV-C	19	Obstructive uropathy	23	6	2	Rejected, 1 yr & 10 yr
V-C	14	Obstructive uropathy	46	4.8	1	Rejected, 1 yr
Mean			32	7.2		

Abbreviations are: GN, glomerulonephritis; IPKD, infantile polycystic kidney disease.

important to renal transplant outcome. To fully probe the significance of transfusion avoidance, we examined not only serial PRA levels but also the strength of discrete anti-HLA Class I antibody specificities, an approach heretofore not reported. Our studies indicated that transfusion withdrawal was associated with a significant decrement in the strength of anti-HLA antibodies in all patients even when the breadth of the antibody response, that is, the %PRA, did not change dramatically. We also observed a down-regulation of nonspecific T cell function following avoidance of transfusions.

Methods

Patients

Five transfusion dependent adolescents, aged 12 to 18 years, undergoing CCPD comprised the study group. Patient data are presented in Table 1. Inclusion criteria, rHuEPO dosage and therapeutic outcome have been reported previously [12]. Four of the five patients had rejected at least one prior renal allograft. Prior to initiation of rHuEPO, the study patients received a mean of 41 blood transfusions (range 23 to 77), over a mean of 46 months (range 21 to 96 months). Each patient had a PRA of at least 40%. rHuEPO was administered for a mean of 13.6 months (range 9 to 18 months). In four of the five patients, rHuEPO was discontinued at the time of renal transplantation; the fifth patient (#V) continues to receive rHuEPO while on dialysis. Following an observation period of at least three months, baseline studies were obtained and rHuEPO begun. Immunological studies were performed at three to six month intervals. Final immunological studies were performed immediately prior to renal transplantation in four patients and at the arbitrary conclusion of the study (15 months) in the patient remaining on dialysis. rHuEPO was supplied by Amgen Inc., Thousand Oaks, California, USA.

The control group was five patients retrospectively matched for age, sensitization status, transfusion requirement and transplant history. Their clinical data are presented in Table 2. They had received a mean of 32 blood transfusions (range 23 to 46), over a period of 43 months (range 24 to 84 months). Humoral assays were performed in both the study and control group; cellular assays were performed in the study group only. The study was approved by The UCLA Human Subjects Protection Committee and written informed consent was obtained prior to initiation of the study.

Immune monitoring—humoral

At three month intervals, aliquots of sera were obtained, frozen at −70°C and stored for assays of humoral sensitization. Anti-Class I HLA antibodies were assayed by the standard long incubation CDC assay, as modified in our laboratory, against a 40 member T-lymphocyte panel [1]. All reagents were obtained from Gibco (Santa Clara, California, USA). Serial dilutions were made with McCoy's 5A medium with 20% fetal calf serum. Briefly, 1 μl of sera or a dilution thereof was aliquoted into four replicate wells of oiled microtiter trays. One μl of T-lymphocytes (previously separated by negative selection using the H4 monoclonal antibody from One Lambda, Los Angeles, California, USA) was adjusted to 3×10^6 cells/ml, added to each well and incubated at 20°C for 60 minutes. Five μl preselected rabbit complement (Pel Freeze, Brown Deer, Wisconsin, USA) was added and incubated for 120 minutes. Following three minute eosin staining and formalin fixation, trays were read independently by at least two of the authors. Significant cytotoxicity was defined as > 50% of cells killed in a given well. The %PRA was expressed as the percentage of the T lymphocyte panel killed in the CDC assay by the undiluted test serum [1].

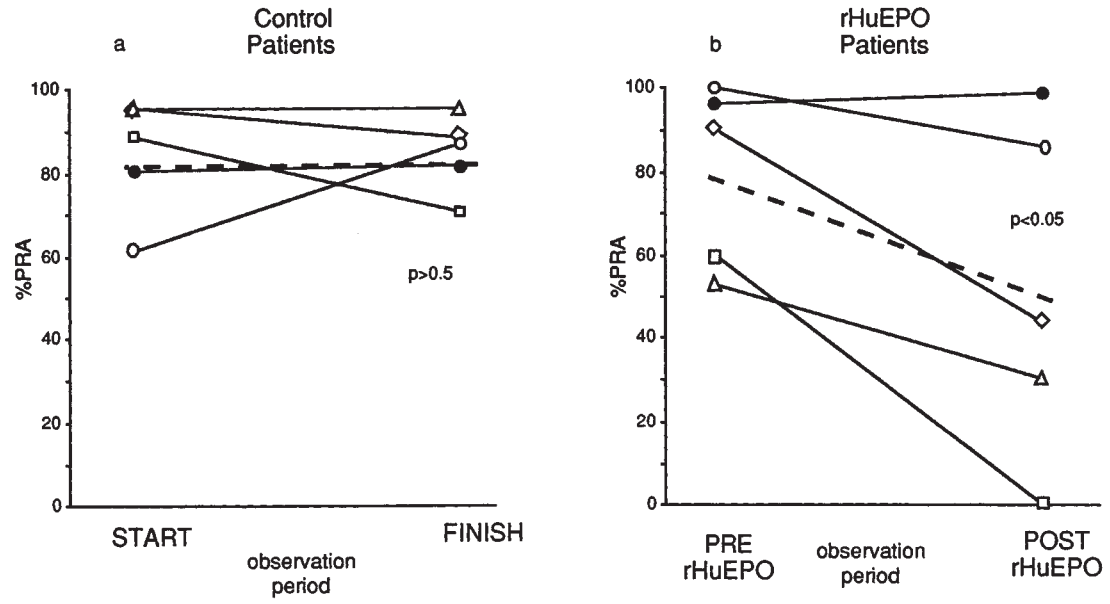

Fig. 1. *Anti-HLA class I antibody as measured by the %PRA.* The interrupted line indicates the mean. (**a**) Control patients at the start and finish of the observation period (mean 18 months) demonstrated no significant change in mean %PRA; 86 ± 15% at the start and 86 ± 9.5% at the finish ($P > 0.5$). (**b**) Patients treated with rHuEPO demonstrated a mean %PRA at the initiation of the rHuEPO of 80 ± 24% that fell to 56 ± 40.6% at the termination of the study ($P < 0.05$). Symbols are patient number: I (○); II (□); III (△); IV (◇); V (●).

For each patient's serum the anti-HLA Class I specificities were determined using the HKB® Software Package installed on an IBM computer, using a correlation coefficient (r value) cutoff of > 0.5 above which a specificity could be assigned [13]. If undiluted sera were too broadly reactive to assign identifiable specificities, dilutions were used to identify all of the strong specificities remaining after dilution [14]. After all specificities were determined, the titer of each anti-HLA class I antibody specificity was defined as the highest dilution killing < 50% of the cells expressing that antigen, that is, the dilution at which there was no significant killing. The titering studies were performed simultaneously on all sera to minimize interassay variation.

Immune monitoring—cellular

Isolation of peripheral blood mononuclear cells. At three to six month intervals 12 ml of heparinized blood was obtained from each of the study patients. Peripheral blood mononuclear cells (PBM) were sterilely isolated by standard density gradient centrifugation using Histopaque (sg 1.077, Sigma, St. Louis, Missouri, USA) [15], washed twice in Hank's Balanced Salt Solution (HBSS) and resuspended at 10^6 cells/ml in RPMI 1640 with glutamine 2 mmolar, 10 mmolar HEPES buffer, 10% human AB serum, penicillin 50 μ/ml and streptomycin 50 μg/ml (Complete RPMI).

PHA stimulation. The mitogen, phytohemagglutinin (PHA; Difco, Detroit Michigan, USA), was dissolved in complete RPMI. 10^5 Responder cells were cultured in a total volume of 200 μl of PHA at final concentrations of 5, 10 and 20 μg/ml in five replicate wells in a 5% CO_2 37°C incubator. At 72 hours a pulse of 1.25 μCu/well ^3H thymidine was added in 50 μl complete RPMI. After incubation for an additional 18 hours, incorporated radiolabel was measured by harvesting using a Multiple Automated Harvesting Unit (Titertek), dried, then counted with a Beckman Liquid Scintilation Counter using Omnifluor (DuPont, Boston, Massachusetts, USA) in toluene as the scintilator. Results were expressed as the total counts/minute (CPM) ± SD.

Allogenic mixed lymphocyte reaction (MLR). The MLR was assayed using autologous as well as allogenic cell stimulators. Allogenic stimulator cells from a frozen donor pool of nine members were selected to represent a broad array of class II antigens. Cells were thawed, washed and suspended at 10^6 cells/ml in complete RPMI. Stimulator cells were irradiated with 2000 rads from a Gammacel (Atomic Energy Canada) ^{135}Cesium source. One hundred microliters of stimulator cells were co-cultured with 100 μl responder cells for 120 hours; the cells were then pulsed, harvested and counted as described in the previous section.

Spontaneous blastogenesis and T cell subsets. The assays for spontaneous blastogenesis and T cell subsets have been described previously [16].

Statistical analysis

Statistical analysis was performed using Wilcoxon's signed-rank test for non-parametric data (titers) and the paired t-test for %PRA and mitogen response from the Statview® Software package. Titers were analyzed after inverse logarithmic transformation.

Results

rHuEPO therapy resulted in correction of anemia in each of the five patients [12]. Four of the five patients received no subsequent transfusions. The fifth (#V) received a transfusion for surgical bleeding nine months after starting rHuEPO.

For a mean of 15 months (range 4 to 36 months) prior to

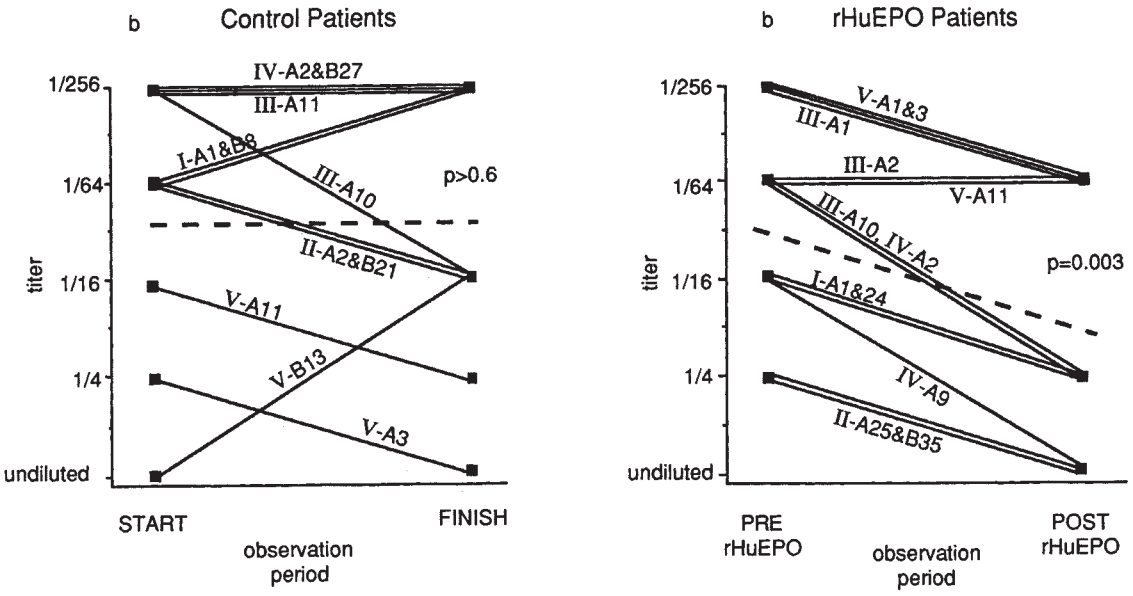

Fig. 2. *Changes in specific anti-HLA titers.* Each line represents a single specificity from a single patient and is labelled with the control patient # first and then the HLA Class I specificity. The interrupted line shows the mean titer. **(a)** In control patients the mean titer remained at (1/32) during the observation period ($P > 0.6$ by Wilcoxon). **(b)** In rHuEPO treated patients the mean titer fell from 1/32 to 1/8 ($P = 0.003$ by Wilcoxon).

initiation of rHuEPO therapy the patients had maintained a %PRA within ± 10% of the value at the start of rHuEPO therapy. Figure 1 compares the baseline PRA at the start of rHuEPO therapy with the PRA after a mean of 13.6 months of rHuEPO therapy. There was a reduction in PRA (Fig. 1B) from the baseline mean value of 80 ± 24% to a mean of 56 ± 40.8% at the termination of the study ($P < 0.05$). The one patient (#V) who maintained a PRA in the 97 to 100% range (Fig. 1B) was the patient who required a transfusion. The control patients continued to require transfusions and experienced no change in %PRA (Fig. 1A). At the beginning of the observation period, the mean PRA was 86 ± 15% and after a mean of 18 (range 8 to 21) months it was 86 ± 9.5% (P = NS).

All Class I anti-HLA antibody specificities and titers were identified, measured and followed in the sera of the rHuEPO and the matched control patients. Each of the five rHuEPO patients showed a reduction of two or more dilutions of at least two anti-HLA antibody specificities. The mean titer of all of the identifiable Class I anti-HLA antibody specificities in the five study patients fell significantly over the course of the study (Fig. 2B). Even patients #III and #V, whose %PRA remained > 80% throughout the course of the study, experienced a titer decrement of two or more dilutions in four of six specificities identified. In the five control patients, 11 Class I anti-HLA antibody specificities were identified in baseline or follow-up studies. As shown in Figure 2A, there was no consistent pattern on change in the titers of these specificities; the titer of three specificities rose, five specificities declined and three remained unchanged. Overall, there was no dominant pattern and no change in the mean titer of the identifiable specificities during the observation period in the control patients (Fig. 2A) ($P >$ 0.6). At the end of the study, only 2 of the 11 identifiable specificities in the control patients were at or below titers of 1:4. By contrast, 7 of the 12 identifiable specificities in the study patients had fallen to 1:4 or below.

The PBM of rHuEPO patients demonstrated a significant decrease in PHA responsiveness during the study period. This was true at all concentrations of PHA tested. A representative set of studies for a PHA concentration of 10 μg/ml is shown in Figure 3. At a PHA concentration of 5 μg/ml, the mean value fell from 5.6 ± 1.8 × 10^5 CPM at the start of the study to 3.0 ± 1.8 × 10^5 CPM at the conclusion; at a PHA concentration of 10 μg/ml, the mean value fell from 6.2 ± 1.9 × 10^5 CPM to 3.9 ± 1.9 × 10^5 CPM (Fig. 3) and at a PHA concentration of 20 ± g/ml, the mean value fell from 5.7 ± 1.1 × 10^5 cpm to 4.0 ± 1.1 × 10^5 CPM ($P < 0.05$ for each PHA concentration level). There were no significant trends in the other assays of cellular immunity.

Four of the five patients in the rHuEPO group terminated the study at the time they received a cadaveric renal allograft. None has yet lost a graft because of rejection. The renal transplants of patients #I, #III and #IV are functioning well with a serum creatinine level of 1.4, 1.0 and 0.6 mg/dl at 21, 15 and 8 months, respectively, after transplant. Patient #II received a cadaveric renal allograft and four months later attained a serum creatinine level of 1.5 mg/dl. Recurrent focal segmental glomerulosclerosis with debilitating nephrotic syndrome required discontinuation of immunosuppression, return to CCPD and allograft embolization. During the observation period of 18 months no control patient received a kidney transplant. In the time since the termination of the study (mean 13 months) only one patient (III-c) has received a kidney. It would have been interesting to determine whether the transplant recipients had historical positive crossmatches against their donors. Unfortunately, there were no viable donor lymphocytes available for us to make this determination.

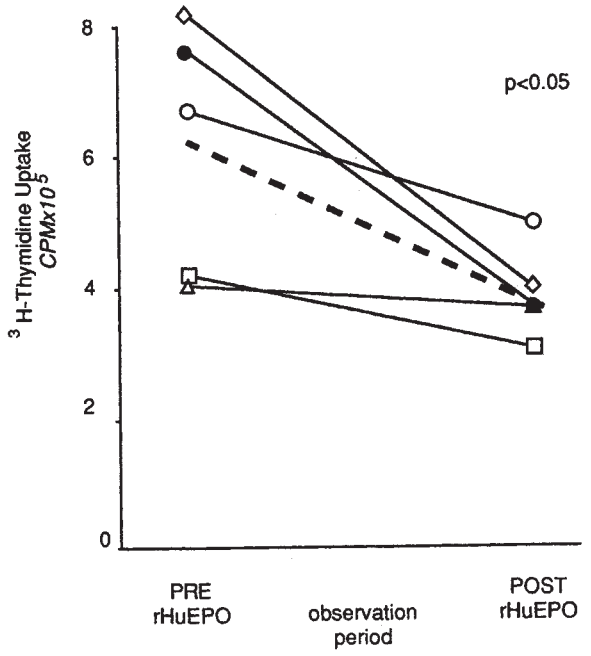

Fig. 3. *Representative example of PHA induced proliferative response at initiation and termination of rHuEPO study.* Proliferation was assessed by uptake of a ^{3}H thymidine pulse and is reported in counts/minute (CPM) $\times$ 10^5. The interrupted line demonstrates the mean CPM fell from $6.2 \pm 1.9 \times 10^5$ to $3.9 \pm 1.9 \times 10^5$ CPM ($P < 0.05$). Symbols are patient number: (△) I; (□) II; (○) III; (●) IV; (◇) V.

Discussion

The importance of blood transfusions in the genesis and maintenance of the highly presensitized state has been intensely debated, particularly in light of the finding that blood transfusions improved allograft survival rates in a dose-dependent manner, that is, increasing numbers of transfusions yielded increasing improvement in graft survival rates [17]. More recently, two developments appear to have dramatically changed the immunological conditioning requirements for those awaiting transplantation. The first is the report by Opelz and the Collaborative Transplant Study documenting that pre-transplant transfusions have lost their primacy as a conditioning regimen [18]. With improvements in post-transplant management and immunosuppression, it now appears that non-transfused recipients have a graft survival rate comparable to that reported for transfused recipients. The second is the clinical availability of rHuEPO. rHuEPO can eliminate the need for blood transfusions in patients on dialysis [10]. In the future, transfusion-induced hyperimmunization should diminish as fewer patients require multiple transfusions.

There are still a large number of hyperimmunized patients currently awaiting renal transplantation [5], and it is unclear how rHuEPO therapy and the avoidance of transfusions will affect them. Deierhoi et al [19] reported a 36 month follow-up of 92 patients, the majority of whom had lost at least one previous renal transplant. They found that the most important risk factor for sustained high levels of anti-HLA sensitization was ongoing transfusions. Norman et al [4] examined the pattern of sensitization that followed five prospective transfusions in non-sensitized untransplanted adults. All but one of the patients who became sensitized with a PRA > 45% demonstrated a reduction after transfusions were curtailed; this reduction occurred over an interval of a few months, and there was complete loss of sensitization by 9 to 18 months. Braun et al [11] followed the %PRA in 16 patients treated with rHuEPO for a mean of 15.4 months. They concluded that rHuEPO allowed patients with low %PRA's to maintain their low level, allowed those with moderate to high %PRA's to experience some fall, but did not affect the %PRA's of the most highly sensitized patients. Scornik et al [20] followed the %PRA of a population of dialysis patients, some of whom had previously rejected a renal allograft. In spite of not receiving recent transfusions, the %PRA of the previously transplanted patients changed little over the observation period, although there was an occasional reduction in antibody concentration as measured by flow cytometry.

These studies suggest that transfusions are an important factor in the maintenance of a sustained high %PRA [19] and that their withdrawal may result in a reduction in the level of sensitization as measured by the %PRA in a significant proportion of highly sensitized patients [4, 11]. Nevertheless, very highly sensitized patients, particularly those who have rejected a previous transplant [20], may not experience a decrease in their %PRA, at least over the time interval studied, in spite of elimination of transfusions.

The results of the present study demonstrate that rHuEPO and concomitant avoidance of blood transfusions led to a fall in %PRA. Indeed, the mean %PRA fell significantly although two of the most highly sensitized patients had no clinically meaningful %PRA decrease. Our study differed from previous ones in that, in addition to %PRA, we examined the sera of rHuEPO-treated and control patients for the titers of discrete Class I anti-HLA antibody specificities. All of the study patients showed reductions of identifiable anti-HLA antibody sensitization despite the fact that four of the five had rejected at least one previous transplant. We found a significant titer reduction in at least two anti-HLA antibody specificities in all five patients studied. This was true even in the patients with the highest %PRA's who experienced either no decrement or a negligible decrement in their level of sensitization. In three of the five patients, the two falling specificities had some cross reactivity; therefore it is possible that the actual number of antibody specificities falling was lower than the 10 of 12 shown in Figure 2B. The control group demonstrates no consistent changes over a comparable observation period. It could be argued that some in the rHuEPO group had lower initial levels of sensitization (that is, %PRA) than the control patients, even though the mean %PRA values were not statistically different. However, the results in the three patients (#III, IV and V) with an initial %PRA of $\geq$ 90% were no different than the results in patients #I and II, who had PRA's of 53% and 60%. Our prospectively studied patient population was small because the Phase I rHuEPO study was limited to five adolescents. Nevertheless, it is reasonable to extrapolate from our data that, given enough time without transfusion, even the most sensitized patients may reduce their Class I anti-HLA sensitization to the point where a crossmatch-negative kidney can become available. At first glance, this latter statement may appear to conflict with the studies of Braun et al [11] and Scornik et al [20], both of which described a population of previously transplanted patients who maintained a very high %PRA in the absence of blood transfu-

sions. However, in both of these studies the authors did not determine the change, if any, in the strength (that is, titer) of the antibodies against discrete Class I anti-HLA specificities (although in one study, antibody strength was assessed against a small number of randomly chosen cells [20]). Our finding of falling titers in all of the rHuEPO patients suggests that the antibody loss is occurring but cannot be detected over relatively short observation periods by the somewhat insensitive %PRA.

Reduction in the titers of Class I anti-HLA antibody specificities will ultimately lead to an increased probability of finding a crossmatch-negative kidney, even if there is only a reduction and not a total disappearance. This is because a reduction in Class I anti-HLA antibody titer, particularly if the antibody in question is broadly reactive, results in a diminution of activity against other members of that particular cross-reacting group [21]. Three of our five patients appeared to have a two titer decrement in at least one broadly reactive anti-HLA antibody specificity (Patients #'s III, IV and V). Thus our finding would appear to have important implications for a large number of highly sensitized dialysis patients awaiting transplantation. If patients can be maintained transfusion-free for a sufficient period of time with rHuEPO, it is reasonable to hypothesize that the frequency of transplantation in highly sensitized patients could increase dramatically.

In addition to the changes in anti-HLA antibody titers, we also found that transfusion avoidance was associated with some evidence of decreased non-specific cell-mediated immunity, as evidenced by a fall in PHA responsiveness. The natural history of the PHA response following rHuEPO treatment is important in view of the studies of Jones et al [6] demonstrating that a high pretransplant PHA response is associated with a significantly poorer short-term renal allograft survival rate than is a low response. This suggests that the long-term elimination of repetitive blood transfusion, and the HLA antigenic stimulation that can occur with rHuEPO therapy may lead to improved allograft survival in highly sensitized patients by allowing T cell responsiveness to down-regulate.

Pfaffl et al [22] reported significant changes in circulating T lymphocyte markers consistent with decreased cell-mediated immune responsiveness after 16 weeks of rHuEPO therapy. They studied 15 patients and found a statistically significant decrease in absolute T cell numbers, CD4 (helper) and CD8 (suppressor) cell numbers, as well as in the CD4/CD8 ratio. We found no significant changes in cellular markers, but this may be due to the small number of patients studied here and a consequent Type II error. This combined functional and phenotypic evidence of a change in the cellular immune system combined with the evidence for a decrease in humoral sensitization, suggests that rHuEPO administration and the resultant elimination of chronic HLA stimulation leads to a down-regulation of immune responsiveness.

With the demonstration by Kerman et al [7] that graft outcome is dependent on the cellular immune responder status of the recipient, our findings are important. In this regard, it is notable that in both the study of Braun et al [11] and our study of patients receiving rHuEPO therapy, allograft loss from rejection was minimal. This is remarkable in view of the high number of highly presensitized patients who received transplants.

The question of whether a high %PRA in itself leads to a poor allograft survival or is just a marker of a more fundamental cellular sensitization remains unanswered. Preliminary data from our laboratory suggest that the highly presensitized state is accompanied by an augmented lymphocyte response to a pool of lymphocytes in the MLR [23]. Regardless of the cellular site at which presentation becomes deleterious for the transplanted kidney, it is likely that transfusion elimination in the highly presensitized patient can only be beneficial to subsequent graft outcome. Indeed, this suggestion has been advanced by others. Norman et al [4] documented 100% two year graft survival in patients sensitized to > 45% PRA by prospective blood transfusions who subsequently 'lost' sensitization after transfusions were discontinued. In this study, the reduction of %PRA was in response to discontinuing HLA stimulation in the form of blood transfusions. Another question is whether there is any active immunological basis for graft outcome improvement in this setting or whether the effect is solely one of antigenic withdrawl. In this regard, the very preliminary demonstration in our laboratory that the sera of some of the rHuEPO patients showed anti-idiotypic antibody activity against Class I HLA antibody may be quite germane [24]. Some authors have presented evidence that the presence of such anti-idiotypic antibodies may be associated with enhanced graft survival [25].

Although this study was performed in adolescents, there is no reason to anticipate that the conclusions to be drawn cannot be extended to highly sensitized patients of any age. We conclude that rHuEPO treatment in dialysis patients can lead to elimination of blood transfusions and consequent reduction in chronic HLA antigenic stimulation. In the dialysis patient this leads to reduction in measurable anti-HLA Class I antibody specificities and may lead to a reduction in %PRA. There are associated functional (mitogen responsiveness) and morphologic (T cell subset [2]) changes suggesting down-regulation of the T cell population. These changes may ultimately lead to a decrease in the waiting time for a cadaveric renal allograft and may facilitate the immunologic success of the allograft. While these studies require confirmation, the potential benefit that rHuEPO may afford the dialysis and prospective renal transplant patient is significant.

Acknowledgments

We thank the volunteers and assistants at the Pediatric Histocompatibility Laboratory for their enthusiasm and help and the UCLA Pediatric Dialysis Nurses for their care of the patients. We are grateful to Amgen for providing the rHuEPO. Portions of this work were presented in abstract form at the American Society of Transplant Physicians Meeting, Chicago, Illinois, May 1989 and at the International Pediatric Nephrology Association Meeting, Toronto, Canada, September 1989. Dr. Grimm is supported by a Fellowship Grant of the Medical Research Council of Canada.

Reprint requests to Dr. Robert Ettenger, Division of Pediatric Nephrology, A2-331 MDCC, UCLA Medical Center, 10833 LeConte Avenue, Los Angeles, California 90024, USA.

References

1. Mittal KK, Mickey MR, Singal DP, Terasaki PI: Serotyping for homotransplantation. XVIII. Refinement of microdrop lymphocyte cytotoxicity test. *Transplantation* 6:913–927, 1968
2. Cheigh JS, Fotino J, Stubenbord WT, Suthanthiran M, Riggio RR, Saal SD: Declining transplantability of prospective kidney transplant recipients. *JAMA* 246:135–139, 1981

3. TING A: HLA matching and crossmatching in renal transplantation, in *Kidney Transplantation, Principles and Practice*, edited by MORRIS PJ, Philadelphia, WB Saunders, 1988, pp. 183–213
4. NORMAN DJ, BARRY JM, BOEHNE C, WETZSTEON P: Natural history of patients who make cytotoxic antibodies following prospective fresh blood transfusions. *Transplant Proc* 17:1041–1043, 1985
5. CECKA JM, CHO L: Sensitization, in *Clinical Transplants 1988*, edited by TERASAKI P, Los Angeles, California UCLA Tissue Typing Laboratory, 1988, pp. 365–374
6. JONES AR, BEWICK M, VOUGHAN RW, BATCHELOR JR: Transformation of lymphocytes from patients awaiting cadaver renal transplants. *Lancet* i:529–531, 1976
7. KERMAN RH, FLOYD M, VAN BUREN CT, KAHAN BD: Improved allograft survival of strong immune responder-high risk recipients with adjuvant anti-thymocyte globulin therapy. *Transplantation* 30:450–454, 1980
8. OPELZ G, GRAVER B, MICKEY MR, TERASAKI PI: Lymphocytoxic antibody responses to transfusions in potential kidney transplant recipients. *Transplantation* 32:177–183, 1981
9. SANFILIPPO F, VAUGHN WK, BOLLINGER RR, SPEES EK: Comparative effects of pregnancy, transfusion, and prior graft rejection on sensitization and renal transplant results. *Transplantation* 34: 360–366, 1982
10. ESCHBACH JW, EGRIE JC, DOWNING MR, BROWNE JK, ADAMSON JW: Correction of the anemia of end-stage renal disease with recombinant human erythropoetin. *N Engl J Med* 316:73–78, 1987
11. BRAUN WE, PAGANINI EP, KLINGMAN L, HEYKA RJ, ZACHARY AA, TERESI G, PROTIVA D, LATHAM D: The impact of recombinant erythropoetin (Epo) on pre-transplant recipient sensitization and transplant outcome. (abstract 8.4-01), 15th Annual American Society for Histocompatibility and Immunogenetics, September 17–21, 1989, Toronto, Ontario, Canada
12. SINAI-TRIEMAN L, SALUSKY IB, FINE RN: Use of subcutaneous recombinant human erythropoetin in children undergoing continuous cycling peritoneal dialysis. *J Pediatr* 114:550–554, 1989
13. GAROVOY MR, MYRBERG SJ, COOPER CM, CARPENTER CB: Computer analysis of presensitization and cross-reacting antibodies. *Transpl Proc* IX:1811–1813, 1977
14. PARK MS, TERASAKI PI, LAU M, IWAKI Y: Sensitization after transplantation, in *Clinical Transplants 1987*, edited by TERASAKI P, Los Angeles, California UCLA Tissue Typing Laboratory, 1987, pp. 393–398
15. BOYUM M: Separation of leukocytes from blood and bone marrow. *Scand J Clin Lab Invest* 21 (sup 97):77–89, 1968
16. ETTENGER RB, BLIFELD C, PRINCE H, BEN-EZER GRADUS D, CHO S, SEKIYA N, SALUSKY IB, FINE RN: The pediatric nephrologist's dilemma: Growth after renal transplantation and its interaction with age as a possible immunologic variable. *J Pediatr* 111:1022–1025, 1987
17. OPELZ G, GRAVER B, TERASAKI PI: Induction of high kidney graft survival rate by multiple transfusion. *Lancet* i:1223–1225, 1981
18. OPELZ G: HLA antigen sensitization: A problem in graft survival. *Transpl Proc* 21 (sup 2):39–41, 1989
19. DEIERHOI MH, SHROYER TW, HUDSON SL, BARGER BO, BARBER WH, CURTIS JJ, JULIAN BA, DIETHELM AG: Sustained high panel reactive antibody levels in highly sensitized patients: Significance of continued transfusions. *Transpl Proc* 21:771–772, 1989
20. SCORNIK JC, SALOMON DR, HOWARD RJ, PFAFF WW: Evaluation of antibody synthesis in broadly sensitized patients. *Transplantation* 45:96–100, 1988
21. OLDFATHER F, MORA A, PHELAN D, KEHL L, FULLER T, ANDERSON C, SICARD G, ETHEREDGE E, RODEY G: The occurrence of cross-reactive "Public" antibodies in the sera of highly sensitized dialysis patients. *Transpl Proc* 15:1212–1215, 1983
22. PFAFFL W, GROSS HJ, NEUMEIER D, NATTERMANN U, SAMTLEBEN W, GURLAND HJ: Lymphocyte subsets and delayed cutaneous hypersensitivity in hemodialysis patients receiving recombinant human erythropoetin. *Contr Nephrol* 66:195–204, 1988
23. GRIMM PC, ETTENGER RB: The highly sensitized (high PRA) state is accompanied by high cellular anti-HLA immune responsiveness in dialysis patients. #42(abs), 9th Annual Meeting of the American Society of Transplant Physicians, May 29–31, 1990, Chicago, Ill, 1990 (Abstract Book, in press)
24. GRIMM PC, ROBERTSON LS, ROBINSON BJ, SEKIYA NM, ETTENGER RB: Serum Anti-idiotype activity developing in dialysis patients treated with recombinant human erythropoetin. #P37(abs), 9th Annual Meeting of the American Society of Transplant Physicians, May 29–31, 1990, Chicago, Ill, 1990 (Abstract Book, in press)
25. REED E, HARDY M, BENVENISTY A, LATTES C, BRENSILVER J, MCCABE R, REEMSTMA K, KING DW, SUCIU-FOCA N: Effect of antiidiotypic antibodies to HLA on graft survival in renal allograft recipients. *N Engl J Med* 316:1450–1455, 1987